

Pathological alterations and prevalence of *Trypanosoma cruzi* in opossums from western Mexico

Alteraciones patológicas y prevalencia de Trypanosoma cruzi en zarigüeyas en el occidente de México

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SUMMARY

There are scarce reports about lesions and pathological alterations by *Trypanosoma cruzi* infections on opossums (*Didelphis virginiana*), considered some of the most important reservoir hosts for *T. cruzi* in western Mexico. After two serological analyses (Serodia and IHA), 12 (24%) of 50 collected opossums in two small towns in western Mexico were positive for the presence of *T. cruzi*. Eight had some kind of organ alterations: four with alterations in only one organ, three in two organs and one with alterations in three organs. Splenomegaly was the most common alteration in the examined opossums. In light of the present findings, it is possible that organ alterations on studied opossums may have been associated with *T. cruzi* infections.

Palabras clave: Chagas disease, opossum, *Trypanosoma cruzi*, organic alterations, Mexico.

RESUMEN

Hay escasos informes sobre lesiones y alteraciones patológicas de las infecciones por *Trypanosoma cruzi* en zarigüeyas (*Didelphis virginiana*), consideradas algunos de los reservorios más importantes para *T. cruzi* en el oeste de México. Utilizando de dos análisis serológicos (Serodia e IHA), 12 (24%) de 50 zarigüeyas capturadas en dos pequeños pueblos en el occidente de México fueron positivas para la presencia de *T. cruzi*. Ocho tenían algún tipo de alteraciones de órganos: cuatro con alteraciones en un solo órgano, tres en dos órganos y uno con alteraciones en tres órganos. La esplenomegalia fue la alteración más común en las zarigüeyas examinadas. A la luz de los actuales resultados, es posible que las alteraciones de órganos en zarigüeyas estudiadas, puedan haber estado asociadas con las infecciones por *T. cruzi*.

Palabras clave: Enfermedad de Chagas, zarigüeyas, *Trypanosoma cruzi*, alteraciones orgánicas, México.

Trypanosoma cruzi, the causative agent of acute and chronic myocarditis (Chagas disease) in humans and dogs, is commonly reported in wildlife reservoir hosts. In Mexico, at least 30 animal species infected by *T. cruzi* have been recorded (Solís-Franco *et al.*, 1997, *Mem. Inst. Oswaldo Cruz.* **92**: 163-164; Velasco-Castrejón & Rivas-Sánchez, 2008, *Bol. Med. Hosp. Inf. Méx.* **65**: 57-79), many of them from the western area of Mexico (including the states of Nayarit, Colima, Michoacán and Jalisco), which is considered one of the most epidemiologically important areas for the transmission of *T. cruzi* to hosts (human or animals) because of the presence of important vector species of triatomines in the

area (Ramsey *et al.*, 2003, En: *Iniciativa para la vigilancia y el control de la enfermedad de Chagas en la República Mexicana*. Eds: Ramsey J. M., Tello-López Á., Pohls J. L. Instituto Nacional de Salud Pública, México). Among those studies devoted to reservoir hosts, few have reported alterations to the organs and lesions of the tissues of hosts infected by *T. cruzi*. Jaime-Andrade *et al.* (1997, *Rev. Soc. Bras. Med. Trop.* **30**: 337-340) reported a scientific curiosity, an acute Chagas cardiopathy case in a polar bear (*Ursus maritimus*) from a zoo in western Mexico. By contrast, Martínez-Ibarra *et al.* (2006, *Acta Bioq. Clín. Latinoam.* Sup. 3, p. 294) briefly reported a more common animal, a specimen of opossum (*Didelphis*

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virginiana), with a digestive alteration (megacolon) from a nearby geographic area. The aim of this study was to increase knowledge of *T. cruzi* infection on the organs and tissues of opossums, a classic Mexican reservoir host of that protozoan.

From January 2007 to September 2008, every two months over two consecutive nights, specimens of opossums were searched in and around two small villages (La Milpilla, 20° 10' N, 103° 24' W; La Villita, 20° 10' N, 103° 23' W) of the municipality of Teocuitatlán de Corona, in the state of Jalisco, an area of western zone of Mexico where human, reservoir hosts as well as vectors have previously been recorded infected by *T. cruzi* (Martínez-Ibarra *et al.*, 2006, *Op. cit.*). Live traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) were used to capture the animals. Traps were baited with a piece of bread covered with canned tuna fish conserved in oil. They were placed at sunset and collected at sunrise since opossums have nocturnal feeding habits (Jiménez-Guzmán *et al.*, 1999, *Pub. Biol. FCB/UANL* **18**: 28-30). Collected animals were transported to the laboratory, where blood tests were undertaken. From each animal, a drop of blood was obtained and directly examined under a microscope for searching for *T. cruzi*. Also, 5 to 10 mL of blood was collected and sera were kept in an icebox before serological testing. Serological tests of Serodia (Latex Particle Agglutination, Fujirebio Diagnostics, Inc., Seguin, TX) (sensitivity of 100%, specificity of 99.8%) and indirect hemagglutination (IHA; Wiener lab, Buenos Aires, Argentina) (sensitivity of 67% and specificity of 99.8%), were applied to evaluate the presence of anti-*T. cruzi* antibodies. In order to establish a possible relationship between infection by *T. cruzi* and pathological alterations, all captured opossums were euthanized by electric shock and their organs obtained. The heart, brain, intercostal muscle, liver, spleen, bladder and esophagus were examined histologically. All tissues were formalin-fixed, embedded in paraffin and stained with hematoxylin and eosin.

Eleven (22%) opossums were positive for the presence of *T. cruzi* by direct blood examination, confirmed when tested with Serodia and with IHA. One additional opossum was positive only by Serodia and HIA and three only by Serodia test.

Eight (66.66%) of those 12 positive opossums for two serological tests to *T. cruzi* had some anatomic/pathological alterations: four specimens had one organ that underwent alteration (cardiomegaly,

megaesophagus, hepatomegaly), three had two affected organs (splenomegaly + megabladder and splenomegaly + cardiomegaly) and one had three affected organs (splenomegaly + cardiomegaly + hepatomegaly). The presence of pseudocysts in the histological samples was not observed. Non anatomic/pathological alterations were recorded between those opossums not positive to *T. cruzi*.

Blood examination proved to be almost as useful for detecting opossums infected with *T. cruzi* as IHA was. Blood examination seems to be a useful method for the detection of infected specimens in field conditions. The percentage of infected *D. virginiana* opossums was low compared with those percentages reported from the same species in other areas: 53.9% in southeastern Mexico (Ruiz-Piña & Cruz-Reyes, 2002, *Mem. Inst. Oswaldo Cruz* **97**: 613-620), 52% in six southwestern and southern states in the USA (Brown *et al.*, 2010, *Vector Borne Zoonotic Dis.* **10**: 757-763) and 37.5% in Louisiana (Barr *et al.*, 1991, *J. Parasitol.* **77**: 624-627). Since infection of opossums seems to occur mainly by oral route (Yaeger, 1971, *J. Parasitol.* **57**: 1375-1376) it is noteworthy the apparent influence of infection rates of the triatomine species present in the study area on the percentages of infected opossums, as previously established for *D. virginiana*, sympatrically collected with *Triatoma dimidiata* in southeastern Mexico (Ruiz-Piña & Cruz-Reyes, 2002, *Op. cit.*). Similarly, *D. virginiana* can be found in the distribution areas of *Triatoma sanguisuga* in southwestern and southern states of the USA, a triatomine species recently found with infection rates from 34.5 to 55.6% (Dorn *et al.*, 2007, *Emerg. Infect. Dis.* **13**: 605-607; Kjos *et al.*, 2009, *Vector-Borne Zoonotic Dis.* **9**: 41-50). By contrast, opossums collected in our study area were collected sympatrically with some specimens of *Meccus longipennis*, which had lower (25.7%) infection rates by *T. cruzi* (Martínez-Ibarra *et al.*, 2010, *Biomedica*, **30**: 140-145). More than 60% of opossums infected by *T. cruzi* developed some kind of potentially deadly pathological alteration (Fidalgo-Álvarez *et al.*, 2003, *Patología Médica Veterinaria*. Universidad de León. León, España). This relationship has been also documented for *D. virginiana* opossums in USA (Barr *et al.*, 1991, *Op. cit.*). More studies on some other populations of opossums are necessary to know more about the relationship between *T. cruzi* infections and pathological alterations on those members of Didelphidae.

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